Contribution to understanding Maf family transcription factors' functions in macrophages in normal and pathological conditions

著者	Dhouha Daassi
発行年	2017
その他のタイトル	正常および病理学的状態におけるマクロファージにおける Mafファミリー転写因子の機能の理解への貢献
学位授与大学	筑波大学 (University of Tsukuba)
学位授与年度	2016
報告番号	12102甲第8308号
URL	http://hdl.handle.net/2241/00147542

氏　　名	Dhouha Daassi
学位の種類	博士（　人間生物学　）
学位記番号	博甲第　8308　号
学位授与年月	平成　29 年　3 月　24 日
学位授与の要件	学位規則　第４条第１項該当（昭和２８年４月１日文部省令第９号）
審査組織	グローバル教育院
学位論文題目	Contribution to understanding Maf family transcription factors' functions in macrophages in normal and pathological conditions (正常および病理学的状態におけるマクロファージにおける Maf ファミリー転写因子の機能の理解への貢献)

	（職名）	（学位）	（氏名）
主　査	筑波大学教授	農学博士	深水　昭吉
副　査	筑波大学教授	医学博士	高橋　智
副　査	筑波大学教授	博士（医学）	千葉　智樹
副　査	筑波大学教授（グローバル教育院）	Ph.D.	Margarete Heck

論文の要旨
Abstract of thesis

Purpose:

Maf family transcription factors share the basic leucine zipper domain (bZIP) and are two classes; large Mafs (MafA, MafB, c-Maf and Nrl) having activation domains at their N-termini and small Mafs (MafK, MafF and MafG) lacking them. Maf proteins can bind to the Maf recognition element (MARE) in the promoter region of their target gene and activate or deactivate expression of the target gene. One of the diseases where macrophages are reported to play big role is the acute kidney injury (AKI) that can be induced by ischemia renal disease (IRD), known by long hospitalization and high mortality. The large Maf transcription factors c-Maf and MafB are expressed in macrophage-lineage hematopoietic cells, but the expression patterns of MafB and c-Maf in macrophage subtypes and tissue-resident macrophages have not been analyzed. Little is known about their functions in the same/different tissue-resident macrophage and/or the same macrophage subtypes. In this work, the applicant aimed to investigate the expression and possible functions of MafB and c-Maf in pathological kidney macrophage of IRD and to elucidate their role in mechanism.

Result:

Mouse lymph nodes, spleens, lungs, and kidneys were subjected to immunohistochemistry using anti-MafB and anti-c-Maf. Both MafB and c-Maf signals were observed in lymph node macrophages. In the splenic macrophages, the MafB signal was detected by anti-MafB, but the c-Maf signal was not detected. No expression of c-Maf or MafB was detected in macrophages in the lung and kidney. Flow cytometry analysis revealed a similar pattern of GFP expression in *Mafb/GFP* knock-in heterozygous mice. To analyze these different expression patterns in greater detail, the applicant examined the expression of MafB and c-Maf by quantitative RT-PCR in different cytokine- or LPS-induced macrophages *in vitro*. *Mafb* expression was induced by IL-10 or IL-4 with IL-13 and was reduced by LPS or GM-CSF. By contrast, *c-Maf* expression was induced by IL-10 and reduced by IL-4 with IL-13 or GM-CSF. These results indicate that MafB and c-Maf have different expression patterns in macrophages, suggesting differences in function. These results suggested that MafB and c-Maf have different expression patterns and different functions in macrophages.

The applicant found that kidney macrophages, initially not expressing MafB and c-Maf, started to express them after 48 hours of IRD. In the present study, the applicant found that in the absence of MafB, mice had worse renal function, as the serum creatinine (CRE) and blood urea nitrogen (BUN) were increased in very high levels. In addition, MafB-deficient mice had a higher mortality rates, high loss of brush border, accumulation of intraluminal debris, enhanced renal tubular injury after ischemia and the $Mac1^{+}F4/80^{low}$ inflamed monocytes were detected. The applicant showed the down regulation of KIM-1 and AIM in conditional knock-out mice (cKO). These results suggest that MafB is important for the recovery of IRD and MafB may be upregulated by IL-4 and IL-13, because these two cytokines are reported to be induced in IRD-induced AKI.

Discussion:

In the present study, the applicant observed remarkably differential expression patterns of MafB and c-Maf in several macrophage subtypes but they are mainly induced in M2, which is an alternatively activated macrophage. In adult tissues, immunohistochemical analyses demonstrated that MafB is expressed in F4/80-positive spleen macrophages, red pulp macrophages that degrade senescent erythrocytes and recycle heme-associated iron. In addition to F4/80-positive lymph node macrophages, which play an important role in immune tolerance by regulating antigen-specific regulatory T cells. This line of evidence indicates that MafB is expressed in macrophages playing homeostatic and immunosuppressive functions. Further analysis using other M2 regulators is required.

In vitro, c-Maf expression was restricted to IL-10-induced macrophages, and the expression of c-Maf in macrophages did not change upon LPS stimulation. Consistent with this result, c-Maf regulates IL-10 expression by directly binding to the promoter region of the IL-10 gene in the presence of LPS. *In vivo*, c-Maf was expressed in F4/80-positive macrophages in the lymph node, but the mechanism by which c-Maf regulates IL-10 *in vivo* is not clear. Further study is required to measure

IL-10 expression in lymph node macrophages using bone marrow reconstitution mice and c-Maf-deficient fetal liver cells.

MafB and c-Maf expression were both decreased in GM-CSF induced macrophages consistently with the fact that GM-CSF deficient mice exhibiting impaired differentiation of alveolar macrophages presented lack of c-Maf and MafB expression. However, in the pathological condition of IRD-induced AKI, kidney macrophages start to express MafB and c-Maf and the resulting kidney injury assessed by CRE/BUN level and histological analysis showed high kidney dysfunction and anatomical damage in absence of MafB. The applicant also found that AIM and KIM-1 were downregulated. Interestingly, AIM was reported to bind with KIM-1 in order to remove the intraluminal debris. Taking together, the applicant's study suggested that the severe IRD-induced AKI phenotype in cKO mice was due to the abrogated intraluminal debris removal and that MafB promotes the recovery or at least attenuates the disease of IRD.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant provided evidence that the expression patterns of MafB and c-Maf vary from non-pathological to pathological macrophages, even within the same tissue-resident macrophages and MafB is mainly induced in M2 condition. In addition, MafB attenuates AKI and is upstream of AIM/KIM signaling pathway. These findings give insight into the molecular mechanisms for the functional contribution of macrophages to renal injuries. Further studies will expect to develop drugs for kidney diseases based on the molecular action of MafB and c-Maf transcription factors, although it is a challenging field of drug discoveries.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 31th January, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.